

Functional verification and screening of protein interacting with the *sIPHB3*

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1 Abstract

2 *sIPHB3* was cloned from *Salix linearistipularis* and mainly
3 expressed in root. The transgenic tobacco was treated with different
4 concentrations of NaCl, NaHCO₃ and H₂O₂, seed germination rate, root
5 length and fresh weigh of transformic tobacco were measured, the results
6 showed that transgenic tobacco was more tolerant to salt, alkali and
7 oxidation than wild-type tobacco. In order to further investigate the

8 molecular mechanism of this gene in *Salix linearistipularis*, we identified
9 24 proteins interacting with sPHB3 by yeast two-hybrid technique. The
10 study of *sPHB3* under abiotic stress can improve our understanding of
11 *PHB3* gene function.

12 Key words: *Salix linearistipularis*, *PHB3*, salt, yeast two-hybrid

13 **Introduction**

14 *Salix linearistipularis* (syn. *S. mongolica*) habitats Inner-Mongolia,
15 Heilongjiang, Jilin, Liaoning, Mongolia and (Far-East) Russia. *Salix*
16 *linearistipularis* is a woody plant that is found in Songnen plain,
17 Heilongjiang, China (Ishida et al., 2009) . *Salix linearistipularis* is a
18 dominant species resistant to saline and plays an important role in
19 maintaining ecological balance and in improving saline soil (Nan et al.,
20 2016).

21 *PHB* was first discovered as a tumor suppressor gene in mammalian
22 cells(Jk et. al., 1989).PHB genes are conserved during evolution (Di et al.,
23 2010; Thuaud et al., 2013). and regulate membrane protein degradation,
24 control cell proliferation and apoptosis (C. Merkwirth et al., 2008;
25 Carsten Merkwirth & Langer, 2009). *PHB3* induced by auxin and shows
26 elevated expression in pericycle cells that give rise to lateral roots(Wang
27 et al.2010). *PHB3* knockout mutants show severe growth defects and
28 have decreased cell division and expansion in the root apex(Wang et al.,

29 2010). *PHB3* impacts SA accumulation and is found in
30 chloroplasts(Seguel et al., 2018). *PHB3* is also present in mitochondria
31 and nuclei, *PHB3* regulates stem cell niche maintenance and cell
32 proliferation during root development in *Arabidopsis* (Huang et al., 2019).
33 *PHB3* is essential to maintain root quiescent center (Kong et al., 2018).
34 The *atPHB3* KO plants had obvious developmental phenotypes with
35 severe growth retardation throughout their development. Germination of
36 *atPHB3* KO seeds was delayed by 1-2 days compared with wild-type
37 (Van Aken et al., 2007). It can be seen that the study of PHB3 protein is
38 very valuable and significant.

39 In this study, *slPHB3* was cloned from *Salix linearistipularis* and
40 transferred into tobacco genome. Physiological index analysis of
41 transgenic tobacco and wild-type tobacco showed that transgenic tobacco
42 plants had stronger salt tolerance, alkaline tolerance, and oxidation
43 resistance than wild-type tobacco. Yeast two-hybrid assay was carried out
44 to further explore the interaction protein with slPHB, there were 24
45 positive clones, among which 17 genes were successfully compared, the
46 rest were unnamed genes and duplicated genes. *slPHB3* enhanced the
47 stress resistance of tobacco plants and provided a basis for the study of
48 *PHB3* introduction in other valuable plants.

49 **Result**

50 **Bioinformatics analysis**

51 The amino acid sequence of the gene cloned from *Salix*
52 *linearistipularis* and PHB3 protein of other plants was compared by
53 DNAMAN software. The amino acid sequence of sPHB3 has a high
54 degree similarity with the amino acid sequence of PHB3 of other
55 plants(Fig. 1). Therefore, the cDNA cloned from *Salix linearistipularis* is
56 named as *sLPHB3*.

57 In order to explore the amino acid sequence homology between
58 sLPHB3 and other plant PHB3, we constructed a phylogenetic tree(Fig. 2),
59 which showed that sLPHB3 is closely related to *PHB3* from *Populus*
60 *trichocarpa*, *Populus euphratica*, and *Populus alba*.

61 **Expression of *sLPHB3* in *Salix linearistipularis* under abiotic stresses**

62 The *sLPHB3* expression level increased until the highest level at 24 h
63 and then decreased gradually under 3 mM H₂O₂ and 5mM NaHCO₃ stress
64 (Fig. 3A).The *sLPHB3* expression level showed its highest value at 12 h
65 under 125 mM NaCl stress (Fig. 3B and C). That means that the
66 stresses influenced the *sLPHB3* gene expression level in *Salix*
67 *linearistipularis* leaves.

68 **Expression of *sLPHB3* gene in transgenic yeast in response to stresses**

69 Transgenic yeast cells growth different under various abiotic stresses
70 were studied (Fig. 4). The *sLPHB3*-transgenic lines grew better in the

71 presence of 1 mM NaCl, 24 mM NaHCO₃ or 3.2 mM H₂O₂ than the
72 control.

73 **Stress tolerance of the transgenic tobacco**

74 The plants grew well in the medium without stress. the transgenic
75 plants severely lost water and wilted than wild-type after stress treatment
76 (Fig. 5).

77 **Screening of *sIPHB3* interacting protein by Yeast two-hybrid**

78 The total of 24 positive gene were obtained from colonies grown on
79 SD/-Trp-Leu-His-Ade+X- α -gal+AbA solid medium turned blue. BLAST
80 these colonies in NCBI , 17 genes were successfully compared (table 1)

81 The function of P-loop containing nucleoside triphosphate
82 hydrolases is related to temperature changes(Zhao L et al, 2019). The
83 function of glycosyl hydrolase family is related to glycosylation (Kotik,
84 M et al., 2020). The glycosyl hydrolase 18 (GH18) and GH19 families
85 are also related to low temperature stress and osmotic stres (Chen J et al.,
86 2018), ATP-dependent protease La is involved in the degradation of
87 abnormally folded proteins and specific regulatory proteins, and regulates
88 protein stability (NAMK A et al.,2020;Tsitsekian D et al., 2019). Lack of
89 Lon hydrolyzed protein causes plant growth retardation and impaired
90 seedling production (Rigas S et al., 2012).The ATP Synthase subunit
91 Beta family is involved in REDOX reactions, mediates protein

92 interactions, and is associated with cold stress (Yang Jh et al.,2020;Zhang
93 Z et al.,2017). Proteome analysis of Date Palm showed that the α and β
94 subunits of ATP synthase changed significantly under salt stress and
95 drought stress (EI Rha et al.,2015). Alpha/beta-hydrolases superfamily
96 functions to catalyze the hydrolysis of ester bonds between fatty acids
97 and glycerol (Xinyi, Zan et al.,2019) .It was involved in delaying the
98 senescence of strawberry fruit at low temperature (B XXa et al.,2015).
99 HSP family functions are related to temperature changes (Li G et
100 al.,2020). However, under NaCl stress, the root-cap length of OSHSP40
101 transgenic seedlings was significantly shorter than that of wild-type
102 seedlings. The results showed that HSP40 was related to salt stress (Wang
103 X et al.,2018). The TIFY family may play crucial and divergent roles in
104 phytohormone crosstalk and plant defense (Liu, X et al.,2020). The
105 TIFY gene responds to abiotic stresses such as jasmonic acid (JA) and
106 salt and drought (Yang, YX et al.,2019) .Glycine decarboxyla plays a
107 major role in photorespiration. Glycine decarboxylase and other proteins
108 increase CO₂ assimilation, vegetative biomass, and seed yield in
109 *Arabidopsis thaliana*.AtGLDP1 is involved in the transition of C-3
110 through C-2 to C4 photosynthesis (Adwy W et al.,2015). Non-intrinsic
111 ABC protein family functions are related to accumulation of metal ions
112 and stability of chloroplast structure (Einav et al.,2010). ATNAP7 is an

113 essential ATP binding site for Arabidopsis embryo development (Xu XM
114 et al.,2004). PAO family functions play a major role in PA catabolism.
115 ScPAO5 is highly responsive to drought stress, while ScPAO1 and
116 CspAO2 are sensitive to changes in nitrogen nutrition (Li M et
117 al.,2020).The transcription level of PaO4 in tomato leaves increased in
118 response to heat stress and cold stress (Upadhyay R K et al.,2020).
119 ABCB4 is a substrate activation regulator of auxin level (Pan Y et
120 al.,2020). Some NAC genes have been identified as candidates for
121 breeding programmes to improve drought resistance in crops (Sepideh et
122 al.,2020). In maize, 13 SNAC transcripts in the SNAC subfamily were
123 responsive to drought stress, and almost all of them in roots and 11 in
124 leaves were upregulated under drought stress (Li L et al.,2015). Salt,
125 osmotic stress and hormone treatment strongly induced the expression of
126 Atstt12 gene (Baek D et al.,2010). Tom3 is thought to have a specific
127 role in the transport of plant iron carriers(Nozoye, T et
128 al.,2015).Proline-rich nuclear receptor coactivator
129 participatescoregulatory protein that modulates transcriptional activation
130 of multiple nuclear receptors(Zhou, D et al.,2000).

131 **Materials and Methods**

132 **Cloning and bioinformatics analysis of *sIPHB3* gene**

133 cDNA was extracted from *Salix linearistipularis*. The forward
134 *sIPHB3F* and reverse primer *sIPHB3R* (Supplementary table S1) were
135 designed according to the *Salix linearistipularis* transcriptome data. The
136 PCR products were ligated to pMD18-T vector (Takara, Tokyo, Japan)
137 and sequenced. The homologous amino acid sequence of *sIPHB3* protein
138 were compared by DNAMAN software, and the phylogenetic tree was
139 constructed by MEGA7.

140 **Real-time quantitative PCR (RT-qPCR) analysis for *sIPHB3*** 141 **expression**

142 *Salix linearistipularis* seeds were sown onto 1/2 MS medium. The
143 seedlings of one-month age were exposed to 3 mM H₂O₂, 150 mM NaCl
144 and 5 mM NaHCO₃ treatments for 0, 6, 12, 24, 36 or 72 h, respectively.
145 The *sIPHB3* expression in leaves under treatments was examined by
146 RT-qPCR analysis, forward and reverse primers in Supplementary table 1.
147 All tests were repeated in triplicate.

148 **Construction of expression vectors and yeast transformation**

149 The coding region of the *sIPHB3* gene was PCR amplified with
150 *Bam*HI sense primer 5'-GGATCCATG-3' and *Xho*I antisense primer
151 5'-CTCGAGTTA-3'. The PCR amplified fragments were digested with
152 *Bam*HI and *Xho*II and then subcloned to the same site of the pYES2

153 expression vector (Clontech, Tokyo, Japan) resulting in pYES2-*sIPHB3*.
154 Then transformed into the competent yeast strain INVSc1 (*S. cerevisiae*)
155 (Clontech) for protein expression in Yeast. Transgenic yeast cells
156 ($OD_{600} = 0.5$), containing pYES2-*sIPHB3* and pYES₂ (control) with serial
157 dilutions (10^0 , 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) were spotted onto YPD agar plates
158 supplemented with 1 M NaCl, 24 mM NaHCO₃ or 3.2 mM H₂O₂,
159 respectively

160 **Acquisition of *sIPHB3* overexpressed tobacco transgenic lines**

161 The *sIPHB3* gene PCR amplified fragment with added with BamHI
162 and XhoI restriction enzymes was obtained by *sIPHB3*BamHI-F and
163 reverse primer *sIPHB3*XhoI-R (Supplement table1) and ligated into
164 pBI121 vector plasmid, The plasmid DNAs of pBI121-*sIPHB3* was
165 transformed into the *Agrobacterium tumefaciens* strain *EHA105* (Takara,
166 Tokyo, Japan), and the *tabacco* was infected . The independent transgenic
167 lines were obtained and verified the expression of *sIPHB3* by RT-qPCR,
168 all templates were tested in triplicate, then used for further analyses.

169 Wild type and transgenic seed were planted to pots containing
170 nutrient-rich soil. After grow up for two months, the pots were irrigated
171 with 50 mL solution of 300 mM NaCl, 300 mM NaHCO₃ or 1.5 M H₂O₂ 3
172 times every 4 days. The pots were covered with a breathable plastic cover
173 to minimize evaporation and keep the concentration of the solution from

174 changing too much. Images of the plants were taken after 12 days of
175 treatment.

176 **Screening of *sIPHB3* interacting protein by Yeast two-hybrid**

177 *sIPHB3* was cloned into pGADT7 vector, and pGADT7-*sIPHB3* was
178 co-transformed into Y₂HGold. After the recombinant plasmids
179 pGADT7-*sIPHB3* were identified by double enzyme digestion, they were
180 transformed into Y₂HGold strain, and then the proteins interacting with
181 pGADT7-*sIPHB3* were screened from cDNA library. Yeast DNA was
182 extracted and sequenced to obtain the interaction gene and protein
183 sequences.

184 **Discussion**

185 Plants have developed specific mechanisms that allow them to detect
186 precise environmental changes and respond to complex stress
187 conditions (Atkinson and P E., 2012). *Methylobacterium* has better
188 survival ability under osmotic stress, which is related to the accumulation
189 of *PHB* in the strain (Woo et al., 2012). In *Arabidopsis thaliana*, *PHB3*
190 coordinates cell division and differentiation in root tip meristems by
191 restricting the ethylene reactive factor (ERF) transcription factor (Kong et
192 al., 2018). An important number of different ROS, including the
193 superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂) are produced under
194 the stresses (Jubany-Marí et al., 2010). Upon *PHB3* loss-of-function, the

195 ROS contents will be out of homeostasis(Huang et al., 2019). Compared
196 to WT, both peroxide (H_2O_2) and superoxide (O_2^-) were overaccumulated
197 in the *PHB3* mutant root meristem(Kong et al.,2018). A *slPHB3* gene was
198 cloned from *Salix linearistipularis*, the *slPHB3* expression level increased
199 under 3 mM H_2O_2 , 125 mM NaCl and 5mM NaHCO_3 stress (Fig. 3). This
200 means the stresses influenced the *slPHB3* gene expression level in *Salix*
201 *linearistipularis* leaves. *slPHB3* may relate to the stresses.

202 In the yeast resistance analysis, the growth of the transgenic strain
203 was better than that of the wild type under 1 mM NaCl, 24 mM NaHCO_3
204 or 3.2 mM H_2O_2 medium treatment, indicating that *PHB3* gene
205 expression added resistant to stress.

206 Transgenic to *PHB3* plants showed higher fresh weight under
207 5mmnahco3 stress. After 2 d of 50 and 100 mM NaCl, the growth of
208 wildtype roots was reduced by 45 and 69%, respectively, whereas root
209 growth was reduced by only 33 and 46% in the mutant (Wang et al.,
210 2010). the function of *PHB3* in root growth under H_2O_2 treatment may
211 be different from that under NaCl treatment(Wang et al., 2010).

212 The wild-type and *lpPHB3* transgenic lines grew well in medium
213 without stress. Under stress induced by 300 mM NaCl, 300 mM NaHCO_3
214 or 2 M H_2O_2 , the wild-type plants died, while the transgenic plants
215 survived; *lpPHB3* transgenic tobacco plants grew well in the medium

216 without stress (Fig. 2D). The results showed that *sIPHB3* transgenic
217 tobacco had stronger resistance to stresses than wild-type tobacco.

218 PHB3 also interacts with a variety of other proteins. In mitochondria,
219 PHB3 forms complexes with other PHB proteins (Van Aken et al., 2016),
220 The formation of the atPHB3-ICS1 complex stabilizes ICS1 to promote
221 SA production (Seguel et al., 2018). In this research, 24 positive clones
222 were obtained (Table 3), Among them, 17 sequences were successfully
223 matched. P-loop containing nucleoside triphosphate hydrolases, HSP, and
224 PAO4 are related to temperature stress. ATP synthase subunit beta is
225 related to cold stress and oxidative stress. ATP synthase epsilon chain,
226 TIF7, SOT12 are related to salt stress, and *NAC13* is related to drought
227 stress.

228 Expression analysis and phenotype analysis showed that *sIPHB3*
229 enhanced tobacco resistance to abiotic stress. The results of yeast
230 resistance analysis and yeast heterozygosity showed that Slphb3 was
231 related to abiotic stress but co-chip assay is needed To determine whether
232 PHB3 interacts with these proteins in cells In order to determine the
233 location of protein interactions, subcellular localization experiments
234 should be performed.

235 **Conclusion**

236 The comparison of physiological indexes between *slPHB3*
237 transgenic tobacco and wild-type tobacco showed that the transgenic
238 plants had higher salt tolerance, alkaline tolerance and oxidation
239 resistance than the wild-type plants. Through the screening of *slPHB3*
240 interaction proteins, 10 of the 17 genes were related to abiotic stress,
241 indicating that *PHB3* gene plays a role in plant stress resistance.

242 Table1. Results of *slPHB3* yeast two-hybrid

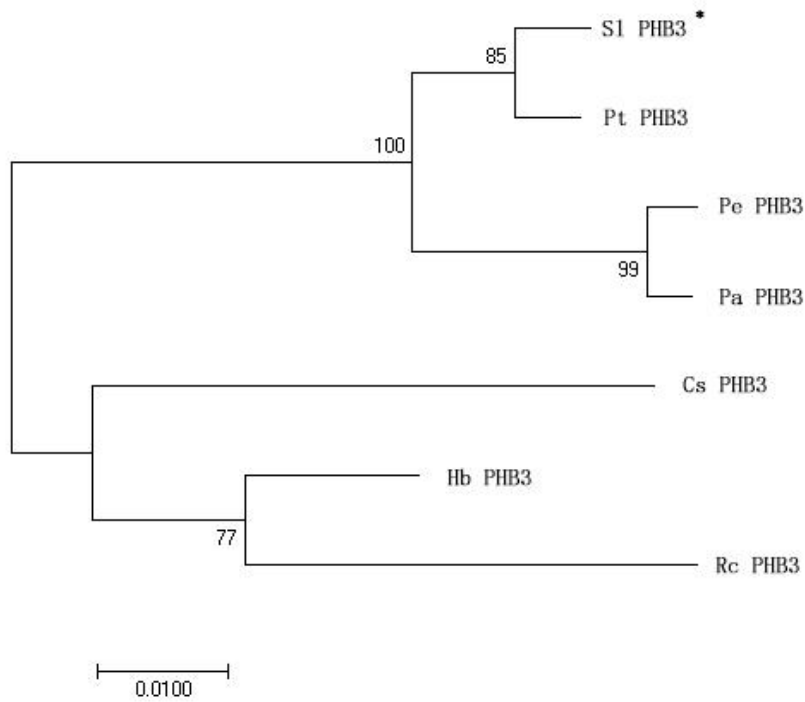
	Gene family
1	P-loop containing nucleoside triphosphate
2	hydrolases glycosyl hydrolase
3	ATP-dependent protease La
4	ATP synthase subunit beta

5	ATP synthase epsilon chain
6	alpha/beta-Hydrolases superfamily
7	DNAJ heat shock family protein
8	TIFY domain/Divergent
9	glycine decarboxyla
10	non-intrinsic ABC protein
11	polyamine oxidase
12	ARABIDOPSIS P-GLYCOPROTEIN
13	Acyl-CoA N-acyltransferases (NAT) superfamily protein
15	NAC domain protein
16	SOT
17	TOM
18	Proline-rich nuclear receptor coactivator

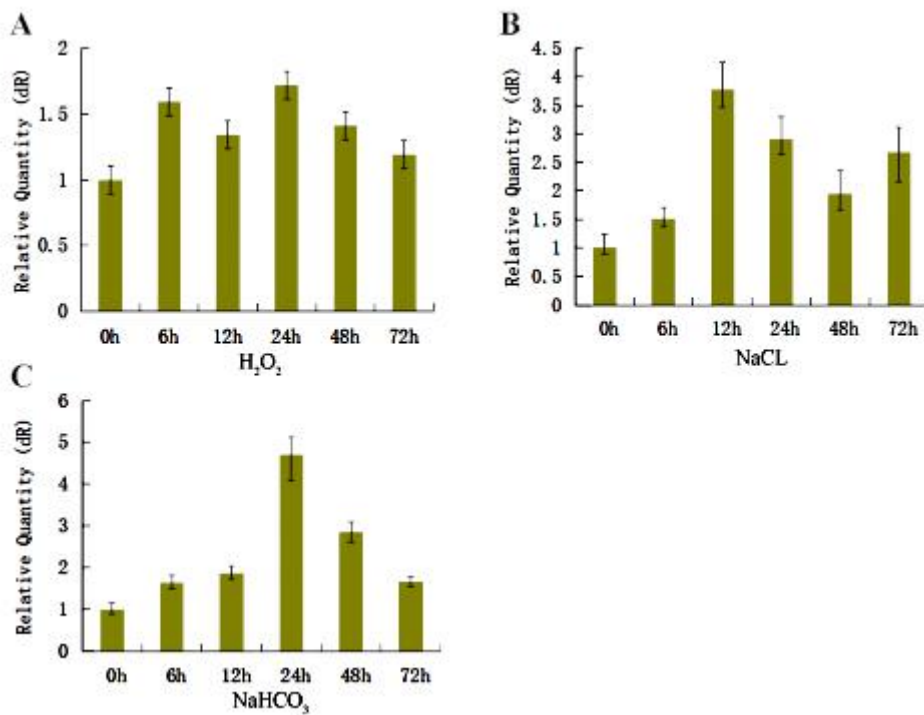
243 Figure Legends



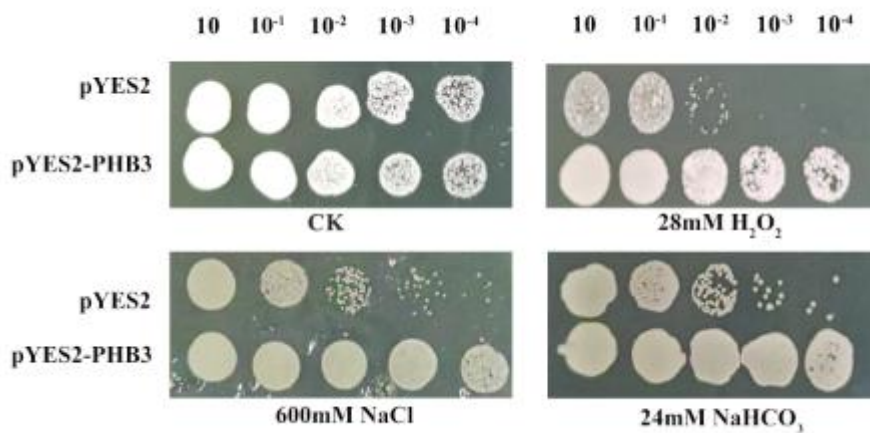
244 Fig1. Alignment of the slPHB3 deduced amino acid sequence with PHB3
 245 proteins from other plant species. The amino acid sequence of the
 246 transcript is similar to that of CsPHB3 Protein, PtPHB3 Protein
 247 (XP_002323792.1), PePHB3 Protein (XP_011045196.1), PaPHB3
 248 protein (TKR74705.1), HbPHB3 Protein (XP_021677719.1), RcPHB3
 249 Protein (XP_002509571.1)



250 Fig.2 *sIPHB3* evolutionary tree analysis. The MEGA7 program was used
 251 for the construction of phylogenetic trees. Bar represents 0.1 amino acid
 252 substitutions per site.



253 Fig3. Real-time quantitative PCR analysis for *sIPHB3* expression in *Salix*
 254 *linearistipularis* under different stresses. (A): Relative expression of
 255 *sIPHB3* at different time under 3mM H₂O₂ stress; (B): Relative
 256 expression of *sIPHB3* at different time under 125mM NaCl stress; (C):
 257 Relative expression of *sIPHB3* at different time under 5mM NaHCO₃
 258 stress.



259 Fig4. Growth of *sIPHB3* transgenic yeast cells under salt stress. Ten-fold
 260 dilutions of yeast cells containing pYES2 (upper line) and
 261 pYES2-*sIPHB3* vector (lower line) were spotted onto solid YPG media
 262 supplemented with the indicated stresses. No treatment is a control (CK).



263 Fig5 The relative stress tolerance of wild-type and transgenic plants (2,
264 # 3, # 4) in reproduction stage was studied. The plants grown on the
265 soil with 1.5 M H₂O₂, 300 mM NaCl or 300mM NaHCO₃ or without
266 (CK).

References

- Adwy W, Laxa M, Peterhansel C. (2015). A simple mechanism for the establishment of C2-specific gene expression in Brassicaceae., *Plant J*,84(6), 1231-1238.<https://doi.org/10.1111/tpj.13084>
- Atkinson NJ and Jain R, Urwin PE (2012). "The interaction of plant biotic and abiotic stresses: from genes to the field." *J. Exp. Bot*,63(10): 3523-3543.<https://doi.org/10.1093/jxb/ers100>
- BAEK DQ, PATHANGE P CHUN JS(2010). A stress-inducible sulphotransferase sulphonates salicylic acid and confers pathogen resistance in arabidopsis. *Plant Cell Environ*, 33(8).<https://doi.org/10.1111/j.1365-3040.2010.02156>.

BAEK D, PATHANGE P, CHUNG J.-S, JIANG J, GAO L, OIKAWA A, HIRAI MY, SAITO K, PARE PW and SHI H. (2010), A stress-inducible sulphotransferase sulphonates salicylic acid and confers pathogen resistance in Arabidopsis. *Plant Cell Environ* , 33: 1383-1392. <https://doi.org/10.1111/j.1365-3040.2010.02156.x>

B XXA , B XM , B HL , B LY , A XS, & B HSA . (2015). Micrnas play an important role in the regulation of strawberry fruit senescence in low temperature. *POSTHARVEST BIOL TEC*, 108, 39-47. <https://doi.org/10.1016/j.postharvbio.2015.05.006>

Chen J, Piao Y, Liu Y, Li X, & Piao Z. (2018). Genome-wide identification and expression analysis of chitinase gene family in brassica rapa reveals its role in clubroot resistance. *PLANT SCI*, 257-267. <https://doi.org/10.1016/j.plantsci.2018.02.017>

267 Chen S, Zhu GQ, He H, Li GL, Ren ZQ, Xu Y, Xu C, Jin SM (2020)A
268 Prohibitin Family Gene (*LpPHB3*) Enhances Salt and Oxidative
269 Stress Tolerance when Overexpressed in *Lilium pumilum*. *Int. J.*
270 *Agric. Biol.* 24, 43–50 .

271 Di C, Xu W, Su Z , Yuan JS.(2010). Comparative genome analysis of
272 PHB gene family reveals deep evolutionary origins and diverse
273 gene function. *BMC Bioinformatics*, 11(S6), S22.
274 <https://doi.org/10.1186/1471-2105-11-S6-S22>

275 El RHA , Al-Malki AL , Abulnaja K O , & Wolfgang R . (2015).
276 Proteome analysis for understanding abiotic
277 stress (salinity and drought) tolerance in date palm (Phoenix
278 dactylifera L.). Int J Genomics,2015,(2015-6-18), 2015,
279 407165.10.1155/2015/407165

280 EINAV, SHIMONI-SHOR, MIRIAM, HASSIDIM, NAOMI,
281 YUVAL-NAEH, et al. (2010). Disruption of nap14, a
282 plastid-localized non-intrinsic abc protein in Arabidopsis thaliana
283 results in the over-accumulation of transition metals and in
284 aberrant chloroplast structures. Plant Cell
285 Environ .<https://doi.org/10.1111/j.1365-3040.2010.02124.x>

286 Huang R , Yang C , Zhang S . (2019). The Arabidopsis *PHB3* is a
287 pleiotropic regulator for plant development. Plant Signal. Behav
288 14(11), 1-5.<https://doi.org/10.1080/15592324.2019.1656036>

289 Huang R, Shu S, Liu M, Wang C, Jiang B, Jiang J, Yang C , Zhang S.
290 (2019). Nuclear Prohibitin3 Maintains Genome Integrity and Cell
291 Proliferation in the Root Meristem through Minichromosome
292 Maintenance 2. PLANT PHYSIOL, 179(4), 1669–1691.
293 <https://doi.org/10.1104/pp.18.01463>

294 Ishida T, Nara K, Ma S, Takano T, & Liu S. (2009). Ectomycorrhizal
295 fungal community in alkaline-saline soil in Northeastern China.

296 *Mycorrhiza*, 19, 329–335.
297 <https://doi.org/10.1007/s00572-008-0219-9>

298 Jk M, Db D, Da S, Jr S, El S, Ck L, Rt D, Mj N. (1989). Isolation of a
299 cDNA that hybrid selects antiproliferative mRNA from rat liver.
300 BBRC, 164(3), 1316–1322.
301 [https://doi.org/10.1016/0006-291x\(89\)91813-5](https://doi.org/10.1016/0006-291x(89)91813-5)

302 Kong X, Tian H, Yu Q, Zhang F, Wang R, Gao S, Xu W, Liu J, Shani, E,
303 Fu C, Zhou G, Zhang L, Zhang X, Ding Z. (2018). *PHB3*
304 Maintains Root Stem Cell Niche Identity through ROS-Responsive
305 AP2/ERF Transcription Factors in Arabidopsis. ,CELL REP 22(5),
306 1350–1363. <https://doi.org/10.1016/j.celrep.2017.12.105>

307 Kotik M , Brodsky K, Halada P, Hana Javrková, Helena Pelantová,
308 Dorota Konvalinková,Pavla Bojarová , Vladimír Křen. (2020).
309 Access to both anomers of rutinoyl azide using wild-type
310 rutinoylase and its catalytic nucleophile mutant. Catal Commun,
311 149:106193 <https://doi.org/10.1016/j.catcom.2020.106193>

312 Kumaran NAM , Karthik M, Kumar V ,Jebasingh T ,Munavar M H ,
313 (2016). Two new mutations in dnaJ, suppress dna damage
314 hypersensitivity and capsule overproduction phenotypes of Δlon ,
315 mutant of escherichia coli, by modulating the expression of clpYq,
316 (hsluv) and rcsA, genes. Gene, 726:
317 144135, <https://doi.org/10.1016/j.gene.2019.144135>

318 Li G , Zhao H , Guo H , Wang Y , Guo X . (2020). Analyses of the
319 function of dnaj family proteins reveal an underlying regulatory
320 mechanism of heat tolerance in honeybee. *Sci. Total Environ*, 716:
321 0048-9697.<https://doi.org/10.1016/j.scitotenv.2020.137036>
322 6

323 Li L, Ma Y, Zhang S , Hao Z , Li X. (2015). Zea mays NAC
324 transcription factor family members: their genomic characteristics
325 and relationship with drought stress. *Research Journal of*
326 *Biotechnology* ,

327 Li M , Lu J , Tao M, Li M , Wan X. (2020). Genome-wide identification
328 of seven polyamine oxidase genes in *camellia sinensis* (L.) and
329 their expression patterns under various abiotic stresses. *Front.*
330 *Plant Sci*,
331 11:544933.<https://doi.org/10.3389/fpls.2020.544933>

332 Liu X, Zhao C, Yang L, Zhang Y, Wang Y, Fang Z, Lv H. (2020).
333 Genome-Wide Identification, Expression Profile of the TIFY
334 Gene Family in *Brassica oleracea* var. *capitata*, and Their
335 Divergent Response to Various Pathogen Infections and
336 Phytohormone Treatments. *Genes*, 11(2), 127. MDPI AG.
337 Retrieved from <http://dx.doi.org/10.3390/genes11020127>

338 McClung JK, Danner DB, Stewart DA, Smith JR, Schneider EL,
339 Lumpkin CK, Dell'Orco RT. and Nuell M. (1989) Isolation of a

340 cDNA that hybrid selects antiproliferative mRNA from rat liver.
341 Biochem. Biophys. Res. Commun. 164,
342 1316–132. [https://doi.org/10.1016/0006-291X\(89\)91813-5](https://doi.org/10.1016/0006-291X(89)91813-5)

343 Merkwirth C, Dargazanli S, Tatsuta T, Geimer S, Lower B, Wunderlich
344 FT, von Kleist-Retzow J-C, Waisman A, Westermann B, Langer T.
345 (2008). Prohibitins control cell proliferation and apoptosis by
346 regulating OPA1-dependent cristae morphogenesis in
347 mitochondria. *Genes Dev.* 22(4), 476–488.
348 <https://doi.org/10.1101/gad.460708>

349 Merkwirth Carsten, Langer T. (2009). Prohibitin function within
350 mitochondria: Essential roles for cell proliferation and cristae
351 morphogenesis. *BBA-MOL CELL RES*, 1793(1), 27–32.
352 <https://doi.org/10.1016/j.bbamcr.2008.05.013>

353 Nan G, Zhang Y, Li S, Lee I, Takano T, Liu S. (2016). NaCl
354 stress-induced transcriptomics analysis of *Salix linearistipularis*
355 (syn. *Salix mongolica*). *BIOL RES-THESSALON* 23(1), 1.
356 <https://doi.org/10.1186/s40709-016-0038-7>

357 Nozoye T, Nagasaka S, Kobayashi T, Sato Y, Uozumi N., Nakanishi, H.,
358 Nishizawa, N. K. (2015). The Phytosiderophore Efflux
359 Transporter TOM2 Is Involved in Metal Transport in Rice. *J Biol*
360 *Chem*, 290(46), 27688–27699.
361 <https://doi.org/10.1074/jbc.M114.635193>

362 Pan Y, Zeng X, Wen, S, Gao X., Liu X, Tian, F, Shang Q. (2020).
363 Multiple ATP-binding cassette transporters genes are involved in
364 thiamethoxam resistance in *Aphis gossypii* glover. *Pestic Biochem*
365 *Physiol*, 167,
366 104558.<https://doi.org/10.1016/j.pestbp.2020.104558>

367 Seguel A, Jelenska J, Herrera-Vásquez A, Marr SK, Joyce MB., Gagesch
368 KR., Shakoor N, Jiang S-C, Fonseca A, Wildermuth M. C,
369 Greenberg JT, Holuigue L. (2018). PROHIBITIN3 Forms
370 Complexes with ISOCHORISMATE SYNTHASE1 to Regulate
371 Stress-Induced Salicylic Acid Biosynthesis in Arabidopsis. *PLANT*
372 *PHYSIO*, 176(3), 2515–2531. <https://doi.org/10.1104/pp.17.00941>

373 Sepideh, Sanjari, Reza, Shirzadian-Khorramabad, Zahra-Sadat, Shobbar,
374 et al. (2019). Systematic analysis of nac transcription factors' gene
375 family and identification of post-flowering drought stress
376 responsive members in sorghum. *PLANT CELL REP* .38,
377 361–376.<https://doi.org/10.1007/s00299-019-02371-8>

378 Shen J , Zou Z , Xing H , Duan Y, Fang,W. (2020). Genome-wide
379 analysis reveals stress and hormone responsive patterns of jaz
380 family genes in *camellia sinensis*. *INT J MOL SCI*, 21(7),
381 2433.<https://doi.org/10.3390/ijms21072433>

382 T. Jubany-Marí S, Munné-Bosch, Alegre L. (2010). Redox regulation of
383 water stress responses in field-grown plants. role of hydrogen

384 peroxide and ascorbate. *PLANT PHYSIOL BIOCH*, 48(5),
385 351-358.<https://doi.org/10.1016/j.plaphy.2010.01.021>

386 Thuaud F, Ribeiro N, Nebigil CG, Désaubry L. (2013). Prohibitin
387 Ligands in Cell Death and Survival: Mode of Action and
388 Therapeutic Potential. *CHEM BIOL*, 20(3), 316–331.
389 <https://doi.org/10.1016/j.chembiol.2013.02.006>

390 Tsitsekian D, Daras G, Alatzas A, Templalexis D, Hatzopoulos P, Rigas S.
391 (2019).Comprehensive analysis of Lon proteases in plants
392 highlights independent gene duplication events. *J Exp Bot*. 2019
393 Apr 12;70(7):2185-2197. <https://doi.org/10.1093/jxb/ery440>.

394 Rigas S , Daras G , Tsitsekian D , Hatzopoulos P . (2012). The
395 multifaceted role of lon proteolysis in seedling establishment and
396 maintenance of plant organelle function: living from protein
397 destruction. *Physiol Plant* 145(1):215-23,
398 <http://dx.doi.org/215-223.10.1111/j.1399-3054.2011.01537.x>

399 Upadhyay RK, Fatima T, Handa AK, Mattoo AK. (2020). Polyamines
400 and Their Biosynthesis/Catabolism Genes Are Differentially
401 Modulated in Response to Heat Versus Cold Stress in Tomato
402 Leaves (*Solanum lycopersicum* L.). *Cells*, 9(8),
403 1749.<http://dx.doi.org/10.3390/cells9081749>

404 Van Aken O, Pečenková T, van de Cotte B, De Rycke R, Eeckhout D,
405 Fromm H, De Jaeger G, Witters E, Beemster GTS, Inzé D, Van

406 Breusegem F. (2007). Mitochondrial type-I prohibitins of
407 *Arabidopsis thaliana* are required for supporting proficient
408 meristem development. *Plant J*, 52(5), 850–864.
409 <https://doi.org/10.1111/j.1365-3113X.2007.03276.x>

410 Wang Y, Ries A, Wu K, Yang A, Crawford NM. (2010). The
411 *Arabidopsis* Prohibitin Gene *PHB3* Functions in Nitric
412 Oxide-Mediated Responses and in Hydrogen Peroxide-Induced
413 Nitric Oxide Accumulation. *Plant Cell* 22(1), 249–259.
414 <https://doi.org/10.1105/tpc.109.072066>

415 Woo SM , Subramanian P , Ramasamy K , Joe MM, Sa TM. . (2012).
416 Eps production, phb accumulation and abiotic stress endurance of
417 plant growth promoting methylobacterium strains grown in a high
418 carbon concentration. *Korean Journal of Soil Science and*
419 *Fertilizer*
420 45(4):361-763.<https://doi.org/10.7745/KJSSF.2012.45.4.572>

421 Wu H, Ye H, Yao R, Zhang T, Xiong L . (2015). Osjaz9 acts as a
422 transcriptional regulator in jasmonate signaling and modulates salt
423 stress tolerance in rice. *Plant Sci*,
424 232:1-12.<https://doi.org/10.1016/j.plantsci.2014.12.010>

425 Wang X., Zhang H., Shao LY., Yan X, Peng H., Ouyang JX , et al.
426 (2018). Expression and function analysis of a rice oshsp40 gene
427 under salt stress. *GENES GENOM* 41(2):175-182.

428 <http://dx.doi.org/10.1007/s13258-018-0749-2>

429 Xinyi Zan, Fengjie Cui, Jianing Shuai Zhou, Yuanda Song. (2019).
430 Novel dual-functional enzyme lip10 catalyzes lipase and
431 acyltransferase activities in the oleaginous fungus mucor
432 circinelloides. J. Agric. Food Chem 67(47):
433 13176–13184.<http://dx.doi.org/13176-13184.10.1021/acs.jafc.9b0>
434 5617

435 Xu XM., & Moller SG. (2004). Atnap7 is a plastidic sufc-like atp-binding
436 cassette/atpase essential for arabidopsis embryogenesis. P NATL
437 ACAD SCI USA,
438 101(24):9143-9148.p.<http://dx.doi.org/9143-9148.10.1073/pnas.0>
439 400799101

440 Yang JH , Williams D, Kandiah E , Fromme P., & Chiu PL . (2020).
441 Structural basis of redox modulation on chloroplast atp synthase.
442 COMMUN BIOL 3(1):482
443 <http://dx.doi.org/482.10.1038/s42003-020-01221-8>

444 Yang Y., Ahammed GJ, Wan C, Liu H, & Zhou Y.. (2019).
445 Comprehensive analysis of tify transcription factors and their
446 expression profiles under jasmonic acid and abiotic stresses in
447 watermelon. Int J Genomic ,
448 2019:1-13.<http://dx.doi.org/10.1155/2019/6813086>

449 Zhanying Zhang, Jinjie Li, Yinghua Pan, Jilong Li, Lei zhou, Hongli Shi,

450 Yawen Zeng, Haifeng Guo, Shuming Yang, Weiwei Zheng,
451 Jianping Yu, Xingming Sun, Gangling Li, Yanglin Ding, Liang
452 Ma, Shiquan Shen, Luyuan Dai, Hongliang Zhang, Shuhua Yang,
453 Yan Guo Zichao L. (2017). Natural variation in *ctb4a* enhances
454 rice adaptation to cold habitats. *Nat Commun.* 8,
455 14788. <http://dx.doi.org/10.1038/ncomms14788>

456 Zhao L., Vecchi G, Vendruscolo M , Krner R , Hartl FU . (2019). The
457 hsp70 chaperone system stabilizes a thermo-sensitive
458 subproteome in *e. coli*. *CELL REP*,28(5):1335-1345,
459 <http://dx.doi.org/1335-1345.e6.10.1016/j.celrep.2019.06.081>

460 Zhou D, Quach KM, Yang C, Lee SY, Pohajdak B, Chen S. (2000).
461 PNRC: a proline-rich nuclear receptor coregulatory protein that
462 modulates transcriptional activation of multiple nuclear receptors
463 including orphan receptors SF1 (steroidogenic factor 1) and
464 ERRalpha1 (estrogen related receptor alpha-1). *Molecular*
465 *endocrinology* (Baltimore, Md.), 14(7), 986–998.
466 <https://doi.org/10.1210/mend.14.7.0480>